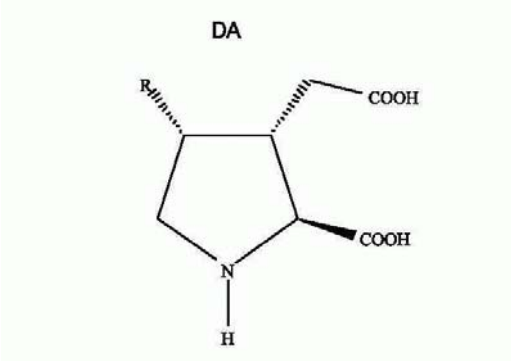
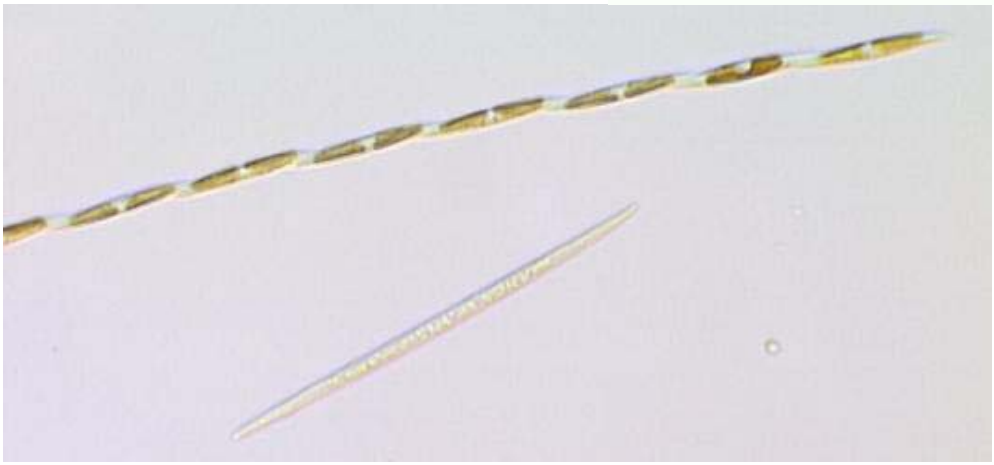
Pseudo-nitzschia spp.

Description: *Pseudo-nitzschia* is a genus of diatom, some members of which produce domoic acid. Some *Pseudo-nitzschia* are toxic and have been found around the northeast and western coast lines of the U.S. and Canada as well as the Gulf of Mexico.



Toxin Produced: Domoic Acid



Syndrome: Amnesic Shellfish Poisoning (ASP)
Amnesic shellfish poisoning (ASP) produces gastrointestinal and neurological effects. Mild cases arise within 24 hours of consumption of contaminated shellfish. Symptoms include nausea, vomiting, diarrhea, and abdominal cramps. In more severe cases neurological symptoms occur which include headaches, hallucinations, confusion, short-term memory loss, respiratory difficulty, seizures, and in extreme cases, death.

Distribution: **West Coast:** Pacific Northwest coast from Canada to mid-California
East Coast: Atlantic Northeast coast of Canada & Gulf of Mexico

Accomplishments: 1994-present

1994: ***Receptor Assays for Domoic Acid and PSP Toxins***
New receptor-based assays for domoic acid and PSP toxins have been found to be useful for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum of exposed humans and animals. These high capacity assays are formatted to contain 96 data points on a 3 x 4" filter card to provide rapid, reliable results and detect all toxin congeners in a manner quantitatively proportional to their toxicity. These assays are anticipated to be used in dock side testing and confirmation of marine toxin exposure in humans and marine animals. *Contact: Fran Van Dolah*

Brain Mapping Studies of Adverse Effects of Ciguatoxins and Domoic Acid
The immediate response gene c-fos has been utilized to map the neuronal pathways activated by marine

toxins in laboratory animals. Using this approach, two major brain regions have been identified to be the targets of amnesic shellfish poisoning; the hippocampus which controls memory processing and the nucleus solitarius which regulates gastrointestinal function. The hippocampus was determined to be irreversibly damaged by this toxin whereas the nucleus solitarius is not. Analogous studies with ciguatoxin have indicated that this toxin activates medial preoptic region of the brain controlling thermoregulation. These studies are being used to better assess the risk of marine toxins to seafood consumers. *Contact: John Ramsdell*

1995: ***Detection of Domoic Acid and PSP Toxin Activity in Algae and Animals***

New rapid and inexpensive receptor-based assays for domoic acid and PSP toxins have been found to be reliable for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum and urine of exposed humans and animals. These assays have been validated against HPLC analytical methods and mouse bioassay. National reference laboratories within the European Community have requested that NMFS offer training workshops on implementing the receptor assays and expressed a desire to initiate collaborative testing programs. These assays are anticipated to be used in dockside testing of shellfish and confirmation of marine toxin exposure in seafood consumers. *Contact: Fran Van Dolah*

Neonates are at High Risk to Domoic Acid Effects

The hazards of marine toxin exposure to seafood consumers have been further elucidated through the use of biomarkers. Two major brain regions have been identified to be the targets of amnesic shellfish poisoning; one controlling memory processing and the other regulating gastrointestinal function. Computer image analysis and reconstruction of this data base has generated three dimensional visualizations of brain damage caused by marine toxin exposure. Neonatal animals have been determined to be ten times more susceptible to amnesic shellfish poisoning. Amnesic shellfish poison (domoic acid) has been determined to be cleared form the serum within hours, and multiple exposures have been found not to change the clearance rate. Domoic acid given to lactating rats passes from the blood to the urine, but is found in very low concentrations in the milk. This indicates that although neonates are highly susceptible to the toxin effects of domoic acid, breast milk may not be a likely route of exposure. These studies are being used to better assess the risk of marine toxins to seafood consumers.

Contact: John Ramsdell

1996: ***Cloned Gluatamate Receptor Used for Domoic Acid Receptor Assay***

Receptor-based assays for marine biotoxins have been further optimized this year. The assay for domoic acid has been modified to utilize a cloned glutamate receptor, and addition of glutamate decarboxylase to sample extracts has been demonstrated to be useful in removing potential interference due to glutamate. The assay has been utilized successfully by the NMFS Seattle Laboratory for field studies on domoic acid production in diatoms. Receptor assays were demonstrated at the NATO Advanced Study Institute on Harmful Algal Blooms, held in Bermuda, May 28-June 6. Feasibility of using receptor assays for shipboard monitoring of algal blooms was demonstrated on a research cruise aboard the R/V Pelican in the Gulf of Mexico, Sept 6-15. *Contact: Fran Van Dolah*

Hazard of Repeated Domoic Acid Exposures

The hazards of marine toxin exposure to specific groups of seafood consumers has been further elucidated, this time investigating the potential hazard to consumers who may receive repeated subsymptomatic exposures to amnesic shellfish poisoning (domoic acid). A battery of tests including toxicokinetics, scored symptomatology, working memory assessment and neurodegeneration analysis and neurodegeneration analysis were used as endpoints to evaluate enhanced toxicity to four repeated exposures to domoic acid. Experiments included both four subsymptomatic and four symptomatic doses and were conducted in two strains of mice, one of which is highly susceptible to drug induced seizures. Repeated exposures did not produced enhanced toxicity in any of the four endpoints, indicating that each

exposure is an independent event that is nonadditive. These results were presented in September to Canadian and U.S. regulatory officials. *Contact: John Ramsdell*

1997: ***Collaborative Testing of Receptor Assays for Marine Toxins***
Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trails. The first of these trails, testing the assay for NSP in oysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY1998. *Contact: Fran Van Dolah*

Domoic Acid Producing Pseudo-nitzschia Identified in the Gulf of Mexico
Pseudo-nitzschia spp. were found to be abundant in Louisiana coastal samples. At shelf sites, these species were present in 70% of all samples and concentrations were minimal in spring, often exceeding one million cells/liter. Among those species identified were the toxic *P. multiseries*, *P. delicatissima*, and *P. pseudodelicatissima.* The DA receptor assay (corroborated by HPLC analyses) detected DA in phytoplankton samples containing *Pseudo-nitzschia* spp. from both estuarine and shelf sites. Future work is aimed at addressing the issues of how environmental factors influence DA production by *Pseudo-nitzschia* spp. in natural populations, and why no outbreaks of DA poisoning have occurred in this region. This project is in collaboration with Dr. Q. Dortch (LUMCON). *Contact: Greg Doucette*

1998: ***Identification of Domoic Acid in California Sea Lions and their Foodweb***
Through the combined use of rRNA probes for *Pseudo-nitzschia* spp., a receptor binding assay and a newly developed liquid chromatography-mass spectrometry method to definitively identify domoic acid, a toxic bloom of *Pseudo-nitzschia australis* occurring this past spring in Monterey Bay, CA was tracked throughout the event. Concurrent use of these novel detection methods, a collaborative effort with the Monterey Bay Aquarium Research Institute, comprises a powerful approach to acquiring near real time data for harmful algal blooms. A time course for the bloom and associated toxin levels was developed, which showed temporal changes in bloom toxicity and the subsequent appearance of a non-toxic species, *Pseudo-nitzschia pseudodelicatissima,* along with a concomitant decline in DA levels. In addition, the *P. australis* bloom was demonstrated to be the source of domoic acid transmitted through planktivorous fish (e.g., anchovies) and ultimately to sea lions in the region, which suffered a mortality event in excess of 50 animals. The latter was the first documentation of naturally incurred DA in mammalian body fluids, providing compelling evidence that the cause of sea lion mortalities was due to DA poisoning.
Contact: Greg Doucette

1999: ***Tandem Mass Spectrometric Identification of Domoic Acid: Absolute Identification of Domoic Acid in California Sea Lions***
A newly developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to provide positive identification of domoic acid in living marine resources and protected species following a toxic bloom of *Pseudo-nitzschia australis* that occurred in spring 1998 in Monterey Bay, CA. The toxic bloom was associated with a sea lion mortality event that resulted in the loss of over 50 animals. The LC-MS/MS method is based upon the detection of highly specific fragmentation products from the collisionally induced dissociation of domoic acid pseudo-molecular ions. The LC-MS/MS method allowed sensitive detection of domoic acid in a variety of matrices ranging from sea lion tissue and excrement to other components of the food web, including planktivorous fish (e.g., anchovies). This is the first utilization of LC-MS for domoic acid identification and is substantial advance in detection methodology because it provides the absolute identification of toxin that is not possible with functional assays or other HPLC-coupled detection methods. *Contact: Mark Busman*

Isolation of a Domoic Acid Producing Diatom From Louisiana Shelf Waters
Screening of eighteen clonal *Pseudo-nitzschia* cultures established by colleagues at LUMCON revealed

production of DA by two of these clones, both identified as *P. pseudodelicatissima* - a species only twice reported as toxic and never verified by mass spectrometry. Both cultures, isolated from LA shelf waters, were confirmed by tandem mass spectrometry to produce DA. Moreover, the patterns of DA production in culture showed the highest cellular toxin levels during exponential growth and the lowest during stationary phase B essentially the opposite of findings reported previously for most other toxic *Pseudo-nitzschia* spp. This pattern is likely due to toxin being excreted into the growth medium during stationary phase; however, since toxin levels are very low, we are currently attempting to reduce our limit of DA detection in seawater to permit the measurement of toxin in the medium. These observations of DA production by exponentially growing cultures have important implications for the toxicity of rapidly growing field populations dominated by this species. *Contact: Greg Doucette*

2000: ***Tandem Mass Spectrometric Identification of Domoic Acid: Identification/Quantitation of Domoic Acid in Scallops From the West Coast of Scotland***

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used in the analysis of domoic acid (DA) in scallops collected during the late 1999 / early 2000 from the West Coast of Scotland. The DA analysis of the scallops was in support of efforts by collaborators at the Scottish Association for Marine Science - Dunstaffnage Marine Laboratory to characterize the dramatic increase in amnesic shellfish poisoning (ASP) on the Scottish west coast. The LC-MS/MS method allowed detection of DA in tissues excised from the scallops. This utilization of LC-MS for domoic acid identification provided the absolute identification of DA in the sampled scallops that would not be accessible from functional assays or other HPLC-coupled detection methods. Results of the LC-MS/MS analyses indicated some of the highest ever recorded levels of DA from shellfish and provide valuable data in support of oceanographic modelling studies concerning the influence of the rapidly expanding Scottish aquaculture industry on the dynamics of harmful algal bloom on the west coast of Scotland. *Contact: Mark Busman*

Determination of Domoic Acid in Mole Crabs (Emerita analoga): A Possible New Indicator Species

We are currently examining the trophic transfer of domoic acid (DA) through the pelagic and benthic food webs in Monterey Bay, California, a site of recurrent *Pseudo-nitzschia* blooms and marine mammal mortalities associated with these HAB events. One aspect of this work, being done in collaboration with CA State Univ. at Monterey Bay and the Univ. of CA at Santa Cruz, is to evaluate use of the mole crab (*Emerita analoga)*, as an indicator species for DA, since the current sentinel organism, the intertidal blue mussel (*Mytilus californianus*), shows minimal or undetectable toxicity during local bloom events. We have developed a new extraction protocol for the determination of DA in mole crabs that yields toxin recoveries of 96.5 $\pm$ 2.9 percent. We have also confirmed by LC-MS/MS that mole crabs accumulated measurable amounts of DA during toxic *Pseudo-nitzschia* bloom events (0.5-10 : g DA/g tissue), while the blue mussel showed no detectable toxin. In addition, the rise and fall of DA toxicity in mole crabs coincided with similar changes in *Pseudo-nitzschia* cell concentrations. Extensive field trials are now underway to further compare the DA toxicity of mole crabs vs. blue mussels and its correlation to the presence of toxic *Pseudo-nitzschia* cells, and to critically evaluate the potential use of mole crabs as the sentinel species for coastal DA events in this area. These data will also be useful in establishing the effectiveness of mole crabs as a vector for transferring DA to higher trophic levels. *Contact: Lisa Hollen*

Identification Of Domoic Acid In Grey Whales And Sea Lions In California Coastal Waters

Using DA receptor assays as a rapid screen and HPLC-MS/MS for chemical confirmation, we were able to confirm that stranded gray whales and sea lions were exposed to DA at concentrations that might be expected to cause adverse effects. We previously demonstrated that domoic acid was the causative agent of a California sea lion mortality event in 1998. This year for the first time, DA poisoning appears to have had widespread impacts on several different marine mammal species with diverse feeding habits and geographic distributions: gray whales stranded in San Francisco Bay in April-May, sea otters that died in the same area during the same timeframe, sea lions stranded in San Luis Obispo County in June-early

July, and sea lions stranded 100 miles farther south in Ventura/Los Angeles Counties in late July. Most of the sea lions were in good body condition, but displayed seisure and scratching activities documented previously in the 1998 sea lion mortality event. Blooms of the DA producing diatom *Pseudonitzschia australis* were found in California coastal waters concurrently with the marine mammal morbidities/mortalities. *Contact: Tod Leighfield*

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